PERSISTENCE OF BACTEROIDES OVATUS UNDER SIMULATED SUNLIGHT IRRADIATION

BY

SHENGKUN DONG

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Environmental Engineering in Civil Engineering
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2013

Urbana, Illinois

Adviser:

Associate Professor Thanh H. Nguyen

Abstract

The persistence of *Bacteroides ovatus*, a human-associated anaerobic fecal contamination indicator bacteria species, was evaluated under simulated sunlight exposure and conditions similar to surface water and seawater. By combining propidium monoazide (PMA) treatment and quantitative polymerase chain reaction (qPCR) detection, the decay rates of *B. ovatus* were determined in the presence of exogenous photosensitizers and salinity up to 39.5 parts per thousand at 27^{o}C. UVB was found to be important for *B.ovatus* decay, averaging a four $\log_{10}$ of decay over 6 h of exposure without the presence of extracellular photosensitizers. The addition of $NaNO_2$, an exogenous sensitizer producing hydroxyl radicals, did not significantly change the decay rate of *B. ovatus*, while the exogenous sensitizer algae organic matter (AOM) slowed down the decay of *B. ovatus*. At seawater salinity, the decay rate of *B. ovatus* is either slower than or as fast as that at freshwater salinity. Exogenous photosensitizers did not accelerate *B. ovatus* decay when being present alone in both low and high salinity solutions. Our study suggests that different *B.ovatus* persistence exists between light and dark treatment: if it is released into either the water or seawater environment in the evening, 50% of it will still be alive by the next morning; if it is released at noon, only 50% will be alive after mere 5 min of full spectrum irradiation on a clear day.

To my parents, relatives, and friends for supporting me from thousands of miles away.

Acknowledgements

I would like to thank my adviser Dr. Thanh H. Nguyen for giving me continuous guidance, support and encouragement throughout my Master research. Thank you to Dr. Pei-Ying Hong at King Abdullah University of Science and Technology for introducing me to molecular biology techniques and providing me with insightful ideas along the way.

I would like to thank all group members in Dr. Thanh H. Nguyen's research group. Special thanks is given to Ofelia Romero-Maraccini and Sahid L. Rosado for introducing me to the fundamentals of solar disinfection.

This research was supported by NSF Career grant #0954501. I would like to express my gratitude to Professor Roderick Mackie in the Department of Animal Sciences and the Functional Genomics Unit in the Biotechnology Center at University of Illinois at Urbana-Champaign.

Table of Contents

1. Introduction

Gastrointestinal illness occurrence in both fresh and coastal water located worldwide has been found to correlate statistically with the presence of fecal indicator bacteria (FIB) such as coliforms and *Enterococci* (Cabelli et al. 1983). The culture-dependent method to enumerate conventional FIB in water bodies for regulatory purposes is commonly used for fecal contamination detection.

However, this method has always been challenged for various reasons. Firstly, because most conventional FIB can enumerate *in vitro*, such as in soil and sediments, the detection of these FIB does not accurately depict the occurrence of a recent fecal contamination event. Monitoring the waterborne illness by cultivating the FIB also underestimates the real number of FIB as it excludes the viable but non-culturable cells (Menon et al. 2003). Secondly, because most conventional FIB are not host-specific and exist in feces of a number of warm-blooded animals, the host origin of fecal contamination cannot be easily identified (Bae and Wuertz 2009b). Thirdly, most FIB require incubation time of 24 h, posing a possibility for people to be exposed to contaminated water before regulatory announcements are made (Hou et al. 2006).

The rapidly emerging microbial source tracking (MST) methods can potentially overcome the disadvantages of using culture-dependent method to evaluate fecal contamination. Among all MST methods, quantitative polymerase chain reaction (qPCR) assays targeting host specific genetic markers for 16S rRNA stands out due to

its specificity, simplicity, and sensitivity. A combination of qPCR and propidium monoazide (PMA) pretreatment has been developed for *Bacteroides* to distinguish between cells with intact membrane and cells with compromised membrane (Bae and Wuertz 2009a). The genera *Bacteroides* has been proposed to replace conventioinal FIB (Fiksdal et al., 1985), because *Bacteroides* are host-specific or host-associated, and the detection of these *Bacteroides* spp. would enable discrimination of the fecal contamination sources (Bernhard and Field 2000b, Fiksdal et al. 1985, Hong et al. 2008). *Bacteroides* spp. are anaerobes and do not propagate easily outside of the host body. Therefore, the presence of these bacteria in the water may be an indication of recent fecal contamination incidents (Haugland 2010).

Recently, US EPA was validating the use of qPCR approach to quantify *Bacteroidales* genetic marker for fecal contamination detection (Haugland 2010). Among the genera *Bacteroides*, *Bacteroides ovatus*, a human associated anaerobic bacterium, has been used as fecal contamination indicator (Bernhard and Field 2000a). However, different host specific *Bacteroidales* have been demonstrated to have various persistence, which is likely due to environmental factors and the stability of DNA markers (Hong et al. 2009). This different persistence would make it challenging to determine the relative contribution of each fecal contamination marker, which is an important factor for MST. Exposure of host specific *Bacteroidales* to sunlight irradiation may be one of the important environmental processes that significantly influence their persistence in surface water (Sinton et al. 2007, Ubomba-Jaswa et al. 2010, Whitman et al. 2004). The DNA marker from

Bacteroidales could persist for days or weeks in naturally sunlit seawater and freshwater environments (Walters and Field 2009, Walters et al. 2009). Such genetic markers have been found to persist longer at lower temperatures and higher salinities (Bell et al. 2009, Okabe and Shimazu 2007), and the persistence of which was identical with or without exposure to natural sunlight (Bae and Wuertz 2012). Since natural sunlight irradiation fluctuates during the day, which might have hindered the precise understanding of *Bacteroidales* persistence in the presence of sunlight irradiation, knowing the accurate correlation between *Bacteroidales* persistence and sunlight irradiation may help in the development of predictive models for MST.

Three fundamental photoinactivation mechanisms have been identified for microorganisms: direct inactivation, indirect exogenous inactivation and indirect endogenous inactivation. For direct inactivation, it refers to the direct damage of the cellular components such as the genome and proteins, by shorter wavelengths of sunlight (Boehm et al. 2009, Davies-Colley et al. 1999). This mechanism has been suggested as the dominant mechanism in bacteria photoinactivation in some cases (Boehm et al. 2009). Indirect endogenous inactivation involves the light absorption by sensitizers that belong to the cell. These sensitizers eventually either pass electrons to other cellular components or create reactive oxygen species that inactivate the cell (Boehm et al. 2009, He and Hader 2002, Santos et al. 2012, van der Meulen et al. 1997). However, it is difficult to experimentally distinguish direct inactivation and indirect endogenous inactivation (Romero-Maraccini et al. 2013, Silverman et al.

2013). Indirect exogenous inactivation usually starts with photo-excitation of the exogenous light sensitizers, such as $NaNO_2$, which reacts with dissolved oxygen and produces reactive oxygen species such as hydroxyl radicals that can oxidize and damage cellular components (Santos et al. 2012). Nevertheless, the contribution of each mechanism to the inactivation of each specific bacterium species is still unclear (Maraccini et al. 2012). Study on inactivation mechanisms of *Bacteroides ovatus*, a human-specific FIB, is particularly lacking.

The objective of this study was therefore to evaluate the decay of *Bacteroides ovatus* under conditions similar to both fresh and coastal seawater, in the presence of controlled simulated sunlight and extracellular natural organic matter from algae. In addition, the study also provides a mechanistic understanding on the decay of a human-associated *B. ovatus*. *Skeletonema costatum* was the algae species chosen due to its wide distribution globally (Serra et al. 1978). The PMA-qPCR approach was used to differentiate and enumerate cells with and without intact membrane. Experiments were designed to answer a number of questions on the effects of sunlight UV irradiation, the roles that exogenous photosensitizers (NO_2^- and AOM), and water salinity would have on the persistence of human-associated fecal indicator *B. ovatus* in the water environment (see Fig 1S in the Supplementary Information).

2. Materials and methods

2.1 Bacteroides ovatus cultivation

Lyophilized *Bacteroides ovatus* received from American Tissue Culture Collection (ATCC 8483) was first suspended in inoculating fluid (Biolog Inc. 72401), followed by inoculation in anaerobic medium(Robert and Bernalier-Donadille 2003) with a modified glucose content of 0.5% (w/v). After the cell suspension reached an OD_{600} of 1, 0.5 mL of the suspension was collected and subsequently inoculated into a serum bottle with 60 ml of anaerobic medium. *B.ovatus* pellets were harvested after 14.5 h of incubation at 37 ^{o}C by centrifugation at 15,557 g for 16 min at 20 ^{o}C. The pellets were washed with 10mM sterilized phosphate buffer solution at pH 8.2 for three times by sequential centrifugation at 15,557g before irradiation experiments.

2.2 Extracellular algae organic matter preparation

Bulk microalgae solution of the species *Skeletonema costatum* propagated in Erdschreiber's medium was purchased from The Culture Collection of Algae at the University of Texas at Austin (UTEX). The bulk solution was subject to centrifugation at 2740 g for 10 min, and the supernatant containing organic cell exudates was collected. The supernatant was filtered through a 1.2 μm cellulose acetate membrane (EPS®, Inc.) to remove algae cells and debris. The filtered supernatant was subjected to dialysis using 3.5k Dalton MWCO membrane (Thermo Scientific SnakeSkin,

88245) against deionized water. The dialysis concentrate, which is referred to as the extracellular algal natural organic matter was harvested when the solution conductivity was below 10 µS/cm. The extracellular algal natural organic matter after dialysis had TOC of 1.94 mg C/L, specific UV absorbance at 254 nm SUVA of 1.03, pH of 6.18, and salinity <1 parts per thousand at 27^{o}C.

2.3 Experimental setup

Eighty ml glass beakers (Pyrex) were used as microcosms, which were wrapped with black tape (3M) to prevent the reflected and deteriorated simulated sunlight screened by the beaker from reaching the liquid inside the microcosm. Mixing was facilitated by a Variomag electronic stirrer set at 130 rpm. Seawater temperatures recorded near King Abdullah University of Science and Technology (KAUST) was 20 ^{o}C in February 2013 and 36 ^{o}C in May 2013. Therefore experiments in this temperature range were conducted. Temperature of the reactors was regulated by a water bath set at 27 ^{o}C. A reactor covered with a piece of aluminum foil was used as the dark control.

Solar exposure was conducted in an Atlas Suntest ® XLS+ photosimulator (Chicago, IL) equipped with a xenon arc lamp set at an irradiance of 400W m^{-2} with a UV special glass filter. A 280 nm cutoff filter (Newport, MA) was placed on top of reactors to prevent liquid loss through evaporation. A 320 nm cutoff filter (Newport, MA) was used to determine the significance of UVB for *B.ovatus* inactivation. An

ILT950 spectroradiometer (International light technologies, MA) was used to measure the irradiance from 280 to 700 nm inside the solar simulator. The fraction of UVB (280 – 320 nm) of the total irradiance from 280 nm to 700 nm produced by the solar simulator was 0.24% (Romero-Maraccini et al. 2013). In comparison, the irradiance fraction of UVB over the total irradiance in natural sunlight during a clear day at 2:10 PM and 2:25 PM on October 21st on KAUST campus was 0.3%. To convert the irradiance (uW/cm^2) to fluence (J/cm^2), cumulative irradiance over 280-700 nm wavelength for a specified duration of time was calculated.

A final cell concentration of 10^9-10^{10} cells/ml was used for all solar exposure experiments. In terms of low salinity situations, for reactors without algal natural organic matter, 10mM sterilized phosphate buffer solution (Fisher Scientific Inc.) was prepared to resuspend the washed *B. ovatus* pellet to 60 ml. Samples were taken intermittently after the initiation of the exposure to the simulated sunlight. 1.2ml of aliquot was drawn out of each microcosm and stored at -20^{o}C for a day before DNA extraction using the Ultraclean Soil DNA Isolation Kit (Mo-Bio, CA) following the manufacturer's instructions. Two batches of samples were collected from a given reactor, one treated with PMA to determine intact cell count and the other was not. Both batches were subsequently enumerated with qPCR assay. For reactors with algal natural organic matter, natural organic matter stock solution was added to the beaker and 500 mM sterilized phosphate buffer (Fisher Scientific Inc.) was used to adjust the pH to 8.2. Ionic strength was then compensated with sodium chloride to 10 mM. For selected experiments, 10 mg/L of $NaNO_2$ was used as synthetic sensitizer of hydroxyl

radicals. Up to five replicates were conducted for each experimental condition.

To mimic the high salinity condition that *B.ovatus* may experience upon being released into the sea, solar exposure experiments were also conducted in artificial seawater. Seawater salinity data recorded at KAUST revealed a salinity of 39.5 parts per thousand (L. Francis 2013). As a result, a stock artificial seawater solution was made accordingly containing 32 g/L of sodium chloride, 0.8 g/L of potassium chloride, 1.3 g/L of calcium chloride and 6.1 g/L of magnesium chloride.

2.4 PMA treatment

PMA (Biotium Inc., CA) was used to count cells with intact membrane. This chemical binds with double stranded DNA in both the extracellular environment and in cells with compromised membrane, preventing subsequent qPCR amplification as previously described in other studies(Bae and Wuertz 2009a, Bae and Wuertz 2012). 2mM of PMA stock solution was prepared using 20% DMSO as the solvent and stored in dark at -20^{o}C until used. For each PMA treatment, 63 µl of the stock solution was added into the 1.2ml sample to achieve 100 µM of the final PMA concentration. The samples containing PMA was incubated for 5 min on a rotary shaker in the dark, followed by exposure to two 500W halogen lamps for 6 min at a distance of 20 cm to photoactivate the dye. During the exposure, the samples were placed on top of the aluminum foil with a tray of ice beneath them to prevent samples from overheating.

2.5 qPCR

384-well clear optical reaction plates (Applied Biosystems®, CA) were used in an ABI PRISM 7500 HT real time quantitative PCR machine (Applied Biosystems®, CA) for amplification and detection. A MicroAmp optical adhesive film (Applied Biosystems®, CA) was used to cover each 384-well clear optical reaction plate to prevent evaporation and contamination. Each 15 µl qPCR reaction mixture contained 2µl of DNA template and 13µl of qPCR mastermix. The mastermix consisted of a mixture of Power SYBR Green (Applied Biosystems®, CA), Bac32F and Bac303R primers (IDT, IA), and molecular biology grade water (Thermo Scientific, MA). Triplicate analysis was done for each sample and a standard curve was generated with serial dilution of Promega pGEM®-T Easy Vector (Madison, WI) harboring the entire 16S rRNA gene from *Bacteroides ovatus*. The thermal cycling parameters include an initial 2 min at 50^{o}C, followed by 10 min at 95 ^{o}C, and 40 cycles of 15 seconds at 95 ^{o}C and 1 min at 60 ^{o}C. A dissociation cycle of 15 seconds at 95 ^{o}C followed by 15 seconds at 60 ^{o}C was done to assess for non-specific amplification. Two samples that contained all the qPCR reagents except for the DNA samples were prepared as negative controls to check for PCR contamination. The detection limit for the Bac32 F and Bac303R primers is 29 copies per reaction; with an amplification efficiency for the set of primers ranging from 90-94%.

2.6 Decay rate calculations

B.ovatus cells with intact membrane will be referred as "live cells" and cells with compromised membrane will be referred as "dead cells" below.

The following pseudo-first decay model was used to fit the data:

$$\mathrm{Ln}\left(\frac{\mathrm{C}}{\mathrm{Co}}\right) = -k_{obs} \times t$$

where t is defined as the time between the beginning of the simulated solar exposure and the sample collection time points. C_0 is the initial copy number of DNA normalized by the mass of the DNA added as the reactant in nanograms, and C is the copy number of DNA at time t (h) normalized by nanograms of DNA added as the reactant. k_{obs}, the pseudo-first decay rate constant was obtained by linear regression fitting. Light screening correction was done on k_{obs} to account for the light absorption and scattering in the solution (Romero et al. 2011). Paired sample t-test was used to determine if the treatment group is significantly different from the dark control group. One-way ANOVA test was done to determine if k_{obs} among more than three treatment groups were significantly different from each other.

Besides the inactivation rate constant, the half-life of *B.ovatus* decay was also calculated for experiments involving artificial seawater based on the following equation:

$$\frac{\mathrm{Ln}\ (0.5)}{-k_{obs}} = t_{1/2}$$

where $t_{1/2}$ is half-life defined as the time for live *B.ovatus* to fall to half of its starting concentration. k_{obs} is the pseudo-first decay rate constant derived from live cell kinetics.

3. Results

3.1 The influence of UV Irradiation on *B.ovatus* decay

For UVA and visible light exposure experiment, there was significant difference between the number of cells with intact membrane in the dark control group and in the group exposed to the simulated sunlight ($p<0.05$, $\alpha=0.05$, $1-\beta=80\%$, Table 1). 1.5 $\log_{10}$ of decay was observed for 6 h of exposure, after which the amount of DNA extracted could not be efficiently amplified and fell below the detection limit of the qPCR for this given primer set. The total *B. ovatus* cells, both intact and damaged ones that were irradiated by full solar spectrum decreased slightly over time.

For the full spectrum irradiation experiment at 27 ^{o}C comprising UVB, UVA and visible light exposure, 4 $\log_{10}$ of decay was observed over 6 h of experiment, with a pseudo-first decay rate constant significantly different from zero ($p<0.05$, $\alpha=0.05$, $1-\beta=80\%$, Table 1). For the dark control, no significant growth or decay was observed (Figure 1). These results suggest that UVB is important for *B. ovatus* decay under simulated sunlight. As a result, to reveal a more prominent decay trend in subsequent experiments, full spectrum irradiation containing UVB, UVA and visible light was used.

Half-life was calculated based on the pseudo-first decay model for live cells. In freshwater irradiated by full spectrum simulated sunlight, half-life was 0.08 h, or 4.8 min. This half-life is significantly different from that in the dark control, which was longer than 9 h.

3.2 The influence of exogenous hydroxyl radicals and algae organic matter on *B.ovatus* decay

Exogenous inactivation mechanism was explored at 27 ^{o}C with full solar spectrum irradiation. Exogenously produced hydroxyl radicals were chosen as they are highly reactive and can reportedly non-specifically attack and oxidize cellular components (Rojanasakul et al. 1993) 0.14mM $NaNO_2$ was added to reactors, which absorbed light at a wavelength of 350nm to produce 0.77$\pm$0.08 fM hydroxyl radicals in freshwater and 0.36$\pm$0.13 fM hydroxyl radicals in seawater. The decay constants measured based on the intact cells were not significantly different for solutions with and without $NaNO_2$ in the freshwater ($p>0.1$, $\alpha=0.1$, $1-\beta=80\%$, Table 1). These results suggest that the presence of exogenous hydroxyl radicals did not significantly impact the inactivation of *B. ovatus* in freshwater (Figure 2).

To simulate some of the actual environmental conditions that *B. ovatus* may be exposed to after being released into the environment, extracted algal natural organic matter having a TOC of 2mg C/L was used to resuspend the washed *B. ovatus* pellets. This TOC value was chosen based on the seawater water quality measurement in Saudi Arabia (L. Francis 2013). The result revealed that at 27^{o}C in freshwater, the presence of AOM slowed down the decay by more than 77% from (9.09$\pm$0.98 hr^{-1}) to (2.06$\pm$0.33 hr^{-1}) compared with the buffer only situation despite producing only a fraction of or even less hydroxyl radicals than that in the solutions containing $NaNO_2$ (Table 2).

Experiments were also conducted to investigate the synergistic effect of $NaNO_2$

and algal natural organic matter on *B. ovatus* decay at 27 ^{o}C. Under these conditions, the k_{obs} value (the pseudo-first decay rate constant) was higher in phosphate buffer simulating freshwater conditions with or without $NaNO_2$ compared with the k_{obs} values obtained in experiments containing both $NaNO_2$ and AOM ($p<0.15$, $\alpha=0.15$, $1-\beta=80\%$, Table 1). Furthermore, slower decay of *B. ovatus* was observed in solutions containing AOM with (4.71 ± 1.86 hr^{-1}) or without $NaNO_2$ (2.06 ± 0.33 hr^{-1}), compared to solution containing only phosphate buffer that simulates freshwater environments (9.09 ± 0.98 hr^{-1}). These observations further indicate that AOM did slow down the *B. ovatus* inactivation despite the presence of exogenous hydroxyl radicals produced.

In terms of half-life, for irradiated experimental settings done with the presence of photosensitizers in the extracellular environment, solution containing AOM has a half-life of 0.34 h, the slowest among the data recorded for low salinity conditions under solar exposure. For the solution containing $NaNO_2$, half-life (0.11 h) is smaller than that of the AOM group but not significantly different than the buffer only group (0.08 h) ($p>0.05$, $\alpha=0.05$, $1-\beta=80\%$). In the dark controls containing only $NaNO_2$, the half-life was recorded to be longer than 12 h. In some dark controls such as the one with AOM, *B. ovatus* even showed a 7% increase in biomass over time.

3.3 The influence of salinity on *B.ovatus* decay

With only the presence of buffered artificial seawater in the extracellular environment, the decay rate constants of *B. ovatus* under full spectrum simulated sunlight were

significantly smaller than that at lower salinity ($p<0.05$, $\alpha=0.05$, $1-\beta=80\%$, Figure 3). Less than 1 $\log_{10}$ of *B. ovatus* decay was observed in the course of 6 h of exposure, with the total cell count changed insignificantly throughout the experiment. In the presence of 0.14mM $NaNO_2$ in artificial seawater, less than 1 $\log_{10}$ of *B. ovatus* decay was recorded, with the fitted k_{obs} value (4.22 ± 0.25 hr^{-1}) not significantly different from that obtained in a photosensitizer free environment in a high salinity setting (3.32 ± 0.38 hr^{-1}) ($p>0.05$, $\alpha=0.05$, $1-\beta=80\%$). This is consistent with the findings in the low salinity experiment. Comparing the k_{obs} values for experiment without extracellular photosensitizers and ones with AOM at high salinity, they did not differ from each other statistically ($p>0.05$, $\alpha=0.05$, $1-\beta=80\%$, Figure 4). In both cases 1 $\log_{10}$ decay of live *B. ovatus* was observed, with the total cell count decreased slightly over time. Mixing 0.14mM $NaNO_2$ and AOM in a high salinity setting yielded unexpected result, with *B. ovatus* decayed faster than when either 0.14mM $NaNO_2$ or AOM was being present alone in the high salinity environment ($p<0.05$, $\alpha=0.05$, $1-\beta=80\%$, Figure 5). The same effect was also observed when comparison was made between high and low salinity conditions, where every other testing parameter was the same (0.14mM $NaNO_2$ and AOM) except for the salinity (Figure 6). Both settings achieved 2 $\log_{10}$ decay of *B. ovatus* in 6 h.

In phosphate buffer condition simulating seawater that was irradiated by full spectrum simulated sunlight, a half-life of 0.21 h was recorded, which was longer than that in low salinity phosphate buffer only condition ($p<0.05$, $\alpha=0.05$, $1-\beta=80\%$, Table 1). This half-life value is statistically the same as that for the phosphate buffer and

$NaNO_2$ irradiated by full spectrum condition in seawater ($p>0.05$, $\alpha=0.05$, $1-\beta=80\%$). Interestingly, for experiment with both $NaNO_2$ and AOM present in a high salinity setting, the half-life was 0.1 h, which was significantly shorter than when either $NaNO_2$ or AOM was being present alone in the high salinity environment ($p<0.05$, $\alpha=0.05$, $1-\beta=80\%$, Table 1). The half-life for the solution containing $NaNO_2$ and AOM at high salinity is also shorter than its low salinity counterpart, which has a slower decay rate constant ($p<0.05$, $\alpha=0.05$, $1-\beta=80\%$, Table 1).

For all dark controls in the seawater experiment, the kinetics of total *B. ovatus* cells had a zero slope ($p>0.05$, $\alpha=0.05$, $1-\beta=80\%$), whereas a slight decrease in the amount of live cells was recorded although a significant difference existed between the decay kinetics of live cells in the dark control and in the solar irradiated experiments ($p<0.05$, $\alpha=0.05$, $1-\beta=80\%$, Table 1). These results indicate that salinity alone did not exert a significant impact on *B. ovatus* persistence in the dark. Irradiation experiments in high salinity water with UVB cut-off was not conducted because UVB irradiation was already identified as an important decay mechanism for *B. ovatus*.

4. Discussion

4.1 Mechanisms of *B. ovatus* decay

From the dark control data, PMA-qPCR did not detect changes in the copy numbers of total cells (i.e., *B. ovatus* with both intact and compromised membranes) and intact cells, suggesting that solution conditions alone did not lead to cell decay or membrane damage. However, for irradiated samples, total cell decayed much less than intact cells, suggesting that irradiation damaged cell membranes. Since it has been reported that medium-pressure UV irradiation damages protein on virus (Eischeid and Linden 2011), it is reasonable to speculate that sunlight UV irradiation does damage to the bacteria membrane., Alternatively, UVB contributes to cell destruction through endogenous inactivation mechanisms that may potentially produce radicals, which in turn damages the cell membrane (Tuveson et al. 1988).

Within the ultraviolet spectrum, UVB played a role in direct inactivation of viral and bacterial cells (Romero et al. 2011, Sinton et al. 2002). In the 6-h time frame of this study, more than 25% decay of total amount of DNA was observed as a result of UVB inactivation. This agrees with other studies, where the abundance of total DNA extracted from cells decreased over time upon solar exposure (Bae and Wuertz 2012). According to previous studies, DNA deformation and decay was observed upon UV irradiation, where inactivation of antibiotic resistant bacteria increased with decreased adjacent thymine sites per genome (McKinney and Pruden 2012). Table 3 depicts the pyrimidine dimer counts for the whole genome of *B. ovatus* constructed from 32

scaffolds, and it was observed that the percentage of thymine dimers per *B. ovatus* genome size was 9.5%. This percentage of thymine dimers was lower than that of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant Enterococci and *Escherichia coli* SMS-3-5, suggesting that *B. ovatus* may be more susceptible to UV irradiation than these three bacterial strains. In addition, bacteria with larger genome sizes were observed to be more susceptible to UV damage, presumably because larger genomes offered more base per sites for UV damage (McKinney and Pruden 2012). The size of the *B. ovatus* constructed from 32 scaffolds was approximately 6.5 Mbp, and was larger than the genome of *Pseudomonas aeruginosa* 01 (i.e., 6.3 Mbp) that was evaluated by McKinney and Pruden (McKinney and Pruden 2012). In their earlier study, *P. aeruginosa* 01 had the largest genome compared to methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant Enterococci and *Escherichia coli* SMS-3-5, and was determined to be most susceptible to UV damage. As such, it is likely to also conclude that *B. ovatus* is equally, if not more, susceptible to UV radiation than *Pseudomonas aeruginosa* 01.

The decay rate of *B. ovatus* with the presence of extracellular photosensitizers at 27 ^{o}C was also evaluated to look into the potential mechanisms that may lead to *B. ovatus* decay. The result was surprising as the inactivation rate constants were statistically the same with and without the presence of extracellular hydroxyl radicals produced by $NaNO_2$, both in low or high salinity settings. Based on these observations, we concluded that for *B. ovatus* under aforementioned conditions, exogenous inactivation was much less significant than endogenous inactivation

mechanisms.

To be more specific about the conditions with extracellular photosensitizers, the presence of exogenous hydroxyl radicals produced by 0.14mM of $NaNO_2$, i.e. up to 0.8 fM of [$^{\bullet}OH$] did not promote significant *B. ovatus* decay (Table 2). In surface water, nitrite is usually converted to nitrate during nitrification. Only in places with incomplete nitrification, nitrite concentration of up to 66 μM was found (Eddy and Williams 1987). In seawater, the concentration of nitrite has been found to be even lower, at 10.5 ± 2nM (Garside 1982). The unrealistically high $NaNO_2$ concentration and [$^{\bullet}OH$] used in our experiment compared to that found in natural water environment did not influence *B. ovatus*'s persistence. Thus, in natural surface water *B. ovatus*'s persistence will not be controlled by environmentally-relevant concentration of [$^{\bullet}OH$].

The presence of AOM in the extracellular environment slowed down the decay of *B. ovatus* in the low salinity setting. This decrease in k_{obs} value suggests that the AOM may protect *B. ovatus* from sunlight irradiation. When *B. ovatus* is discharged into the freshwater environment where AOM is also present on a sunny day, 50% of the total live cells will be lost within 20 min, persisting longer than that without AOM. Our observations suggest that in pockets of freshwater directly exposed to wastewater treatment plants discharge, it is anticipated that the AOM TOC would be higher than 1.94 mg C/L and would facilitate a longer persistence of *B. ovatus* for subsequent MST. When *B. ovatus* is released into seawater with AOM, *B. ovatus* cells have a half-life of 11.4 min, which is essentially the same as the half-life for the seawater

only condition (12.6 min).

A possible explanation to account for a longer persistence of *B. ovatus* in seawater may be due to AOM partially covering the cell surface (thus the endogenous sensitizers), attenuating the solar irradiation that reaches the cell to produce endogenous reactive oxygen species. This mechanism was observed to have been weakened by the presence of high salinity, especially chloride ions, which are believed to be able to scavenge hydroxyl radicals (Rubio et al.).

The results presented here suggest that for the decay rate constants in freshwater conditions, they were significantly lower in the presence of AOM and therefore persist longer, regardless of the presence of hydroxyl radicals. This is consistent with the observation aforementioned, which discovered that for *B. ovatus* exogenous inactivation was much less significant than endogenous inactivation mechanisms. At high salinity, when combining both AOM and hydroxyl radicals produced by $NaNO_2$, the synergistic effect of AOM, hydroxyl radicals, and high salt concentration contributed to the faster decay than that without $NaNO_2$ ($p<0.05$, $\alpha=0.05$, $1-\beta=80\%$, Table 1). Although this synergistic effect means a shorter half-life, it translates to only a difference of 4.2 min. This short time difference may not be apparent when performing actual MST on-site.

4.2 Implication for MST

The experiment results revealed that at 27 ^{o}C, *B. ovatus* could survive in either sea or

freshwater under full spectrum simulated sunlight for up to 6 h and still be reliably detected by the PMA-qPCR approach. This means that in freshwater without the presence of light sensitizers, given the chance for *B. ovatus* to be released in the morning, 50% of *B. ovatus* with intact membrane will still be alive after 4.8 min of solar exposure , after which the decay will slow down and eventually remain detectable after 6 h. In the seawater, it will take 12 min for 50% of the total *B. ovatus* to die under the same solar intensity, slightly longer than that in freshwater. Again, 6 h after its release into the seawater, it was still above the detection limit. This is an important finding because it indicates that during a clear sunny day without cloud, finding intact *B. ovatus* cells in water bodies where there is low concentration of photosensitizers provides information on the occurrence of a fresh fecal contamination within the past 6 h. All of this information is crucial for fecal contamination tracking. These findings contradicts a previous finding, in which Bae and Wuertz concluded that natural sunlight did not significantly impact the persistence of *Bacteroidales* in seawater (Bae and Wuertz 2009b). However, both studies yielded similar *B. ovatus* decay trend at the early stage of the solar irradiation experiment (<6 h) (Bae and Wuertz 2012). In Bae and Wuertz's study, this stage corresponds to the first exponential stage. The decay in our study may be contributed to the constantly high irradiance set on the solar simulator, whereas the Bae and Wuertz's study used natural sunlight, which might have experienced temperature fluctuations, insufficient solar radiation transmittance during the course of the study. Another possibility for the observed discrepancy is that since fluence was not given,

Bae and Wuertz might have intermittently used UVA plus visible light and UVB plus UVA plus visible light, due to the potential overcast weather that might have appeared during the experiment that lasted for many days. Since we have found in our study that UVA plus visible light did not have a significant impact on *B. ovatus* persistence, this may help to explain the longer persistence of bacteria in Bae and Wuertz's study.

For dark controls, the half-life of *B. ovatus* ranges from 9 to 13 h in low salinity settings, and 16 to 34 h in high salinity settings. This means if *B. ovatus* is released in the evening into either fresh or seawater environment, at least 50% of it will still be alive by the next morning. In recent studies evaluating the short-term changes in recreational water quality, researchers demonstrated that monitoring the conventional fecal indicators at different time of the day would result in different test outcomes (Stapleton et al. 2009, Wyer et al. 2010). As a result, the obtained decay kinetic data in this study may help predict live *B. ovatus* cell biomass after its release into the water environment in both the morning and at night time, in order to back-calculate the original amount of biomass upon release.

In terms of the decay rate of *B. ovatus* cells and *B. ovatus* total DNA, our study concludes that at the same fluence value exerted by the solar simulator, for the phosphate buffer only situation in freshwater, *B. ovatus* cells with intact membranes decayed much faster than the *B. ovatus* total DNA, with the former reaching more than 5 $\log_{10}$ of decay and the latter reaching less than 1 $\log_{10}$ of decay. This agrees with Mckinney and Pruden's finding, which stated that the genes from antibiotic resistance bacteria require more UV doses than that to inactivate host bacterial cells

(McKinney and Pruden 2012). Another study done by Walters et al. also concluded similarly that a *Bacteroidales* human-specific DNA marker decayed faster in light-exposed reactors (Walters et al. 2009). As a result, it can be concluded that the persistence of total *B. ovatus* DNA is more than 100 fold longer than that of the viable cell portions, in terms of the half-life. This would imply that the currently proposed method by US-EPA to indicate fecal pollution, which is based on the qPCR enumeration of the total DNA of *Bacteroides*, might still be limited in its efficacy to determine recent fecal contamination events.

5. Conclusions

- DNA degradation for *B. ovatus* under full spectrum sunlight irradiation was observed, the persistence of total *B. ovatus* DNA is more than 100 fold longer than that of the viable cells in terms of the half-life.
- UVB was important for the persistence of intact *B. ovatus* in both fresh and seawater, and was able to decrease the half-life of *B. ovatus* in fresh water by a maximum of 12.73 h and 33.95 h in sea water. *B. ovatus* in general persisted longer in sea water than in fresh water under same conditions with full spectrum UV radiation.
- In the fresh water, the persistence of *B. ovatus* under simulated full spectrum sunlight was enhanced by AOM by 4 fold in terms of the half-life. Extracellular hydroxyl radicals produced by 0.14 mM $NaNO_2$ did not have observable effect on *B. ovatus* persistence. In addition, lowest persistence (0.09 h of half-life) was observed in solutions containing no AOM with or without $NaNO_2$.
- In the artificial seawater, neither AOM nor extracellular hydroxyl radicals had significant effect on *B. ovatus* persistence. However, the synergistic effect of AOM and extracellular hydroxyl radicals produced by $NaNO_2$ led to the lowest *B. ovatus* persistence (0.1 h of half-life).
- In dark controls, *B. ovatus* persisted for long periods of time ranging from 9 h to 34 h depending on the solution that *B. ovatus* was exposed to. Based on the dark control results, if *B. ovatus* is released into either the freshwater or seawater environment in the evening, 50% of it will still be alive by the next morning. Based

on the data from irradiation experiments, if *B. ovatus* is released in the morning, only 50% will be alive after mere 5 min exposure to full spectrum sunlight on a clear day.

6. References

Bae, S. and Wuertz, S. (2009a) Discrimination of viable and dead fecal Bacteroidales bacteria by quantitative PCR with propidium monoazide. Appl Environ Microbiol 75(9), 2940-2944.

Bae, S. and Wuertz, S. (2009b) Rapid decay of host-specific fecal Bacteroidales cells in seawater as measured by quantitative PCR with propidium monoazide. Water Res 43(19), 4850-4859.

Bae, S. and Wuertz, S. (2012) Survival of Host-Associated Bacteroidales Cells and Their Relationship with Enterococcus spp., Campylobacter jejuni, Salmonella enterica Serovar Typhimurium, and Adenovirus in Freshwater Microcosms as Measured by Propidium Monoazide-Quantitative PCR. Applied and Environmental Microbiology 78(4), 922-932.

Bell, A., Layton, A.C., McKay, L., Williams, D., Gentry, R. and Sayler, G.S. (2009) Factors influencing the persistence of fecal Bacteroides in stream water. J Environ Qual 38(3), 1224-1232.

Bernhard, A.E. and Field, K.G. (2000a) Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. Appl Environ Microbiol 66(4), 1587-1594.

Bernhard, A.E. and Field, K.G. (2000b) A PCR assay To discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA. Appl Environ Microbiol 66(10), 4571-4574.

Boehm, A.B., Yamahara, K.M., Love, D.C., Peterson, B.M., McNeill, K. and Nelson, K.L. (2009) Covariation and Photoinactivation of Traditional and Novel Indicator Organisms and Human Viruses at a Sewage-Impacted Marine Beach. Environmental science & technology 43(21), 8046-8052.

Cabelli, V.J., Dufour, A.P., McCabe, L.J. and Levin, M.A. (1983) A Marine Recreational Water Quality Criterion Consistent with Indicator Concepts and Risk Analysis. Journal (Water Pollution Control Federation) 55(10), 1306-1314.

Davies-Colley, R.J., Donnison, A.M., Speed, D.J., Ross, C.M. and Nagels, J.W. (1999) Inactivation of faecal indicator micro-organisms in waste stabilisation ponds: interactions of environmental factors with sunlight. Water Research 33(5), 1220-1230.

Eddy, F.B. and Williams, E. (1987) Nitrite and freshwater fish. Chemistry and Ecology 3(1), 1-38.

Eischeid, A.C. and Linden, K.G. (2011) Molecular indications of protein damage in adenoviruses after UV disinfection. Appl Environ Microbiol 77(3), 1145-1147.

Fiksdal, L., Maki, J.S., LaCroix, S.J. and Staley, J.T. (1985) Survival and detection of Bacteroides spp., prospective indicator bacteria. Appl Environ Microbiol 49(1), 148-150.

Garside, C. (1982) A chemiluminescent technique for the determination of nanomolar concentrations of nitrate and nitrite in seawater. Marine Chemistry 11(2), 159-167.

Haugland, R.O., K.; Dufour, A.P. (2010) Method B: Bacteroidales in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay

He, Y.Y. and Hader, D.P. (2002) Involvement of reactive oxygen species in the UV-B damage to the cyanobacterium Anabaena sp. J Photochem Photobiol B 66(1), 73-80.
Hong, P.-Y., Wu, J.-H. and Liu, W.-T. (2009) A high-throughput and quantitative hierarchical oligonucleotide primer extension (HOPE)-based approach to identify sources of faecal contamination in water bodies. Environmental Microbiology 11(7), 1672-1681.
Hong, P.Y., Wu, J.H. and Liu, W.T. (2008) Relative abundance of Bacteroides spp. in stools and wastewaters as determined by hierarchical oligonucleotide primer extension. Appl Environ Microbiol 74(9), 2882-2893.
Hou, D., Rabinovici, S.J.M. and Boehm, A.B. (2006) Enterococci Predictions from Partial Least Squares Regression Models in Conjunction with a Single-Sample Standard Improve the Efficacy of Beach Management Advisories. Environmental Science & Technology 40(6), 1737-1743.
Kohn, T., Grandbois, M., McNeill, K. and Nelson, K.L. (2007) Association with natural organic matter enhances the sunlight-mediated inactivation of MS2 coliphage by singlet oxygen. Environ Sci Technol 41(13), 4626-4632.
L. Francis, N.G., A. Al-Saadi, F. Wali, S.P. Nunes, G. L. Amy (2013) Performance evaluation of the DCMD desalination process under different operating conditions. Journal of Membrane Science.
Maraccini, P.A., Ferguson, D.M. and Boehm, A.B. (2012) Diurnal Variation in Enterococcus Species Composition in Polluted Ocean Water and a Potential Role for the Enterococcal Carotenoid in Protection against Photoinactivation. Applied and Environmental Microbiology 78(2), 305-310.
McKinney, C.W. and Pruden, A. (2012) Ultraviolet disinfection of antibiotic resistant bacteria and their antibiotic resistance genes in water and wastewater. Environ Sci Technol 46(24), 13393-13400.
Menon, P., Billen, G. and Servais, P. (2003) Mortality rates of autochthonous and fecal bacteria in natural aquatic ecosystems. Water Research 37(17), 4151-4158.
Okabe, S. and Shimazu, Y. (2007) Persistence of host-specific <i>Bacteroides–Prevotella</i> 16S rRNA genetic markers in environmental waters: effects of temperature and salinity. Applied Microbiology and Biotechnology 76(4), 935-944.
Robert, C. and Bernalier-Donadille, A. (2003) The cellulolytic microflora of the human colon: evidence of microcrystalline cellulose-degrading bacteria in methane-excreting subjects. FEMS Microbiol Ecol 46(1), 81-89.
Rojanasakul, Y., Wang, L., Hoffman, A.H., Shi, X., Dalal, N.S., Banks, D.E. and Ma, J.K. (1993) Mechanisms of hydroxyl free radical-induced cellular injury and calcium overloading in alveolar macrophages. Am J Respir Cell Mol Biol 8(4), 377-383.
Romero-Maraccini, O.C., Sadik, N.J., Rosado-Lausell, S.L., Pugh, C.R., Niu, X.-Z., Croué, J.-P. and Nguyen, T.H. (2013) Sunlight-Induced Inactivation of Human Wa and Porcine OSU Rotaviruses in the Presence of Exogenous Photosensitizers. Environmental Science & Technology 47(19), 11004-11012.
Romero, O.C., Straub, A.P., Kohn, T. and Nguyen, T.H. (2011) Role of Temperature and Suwannee River Natural Organic Matter on Inactivation Kinetics of Rotavirus and Bacteriophage MS2 by Solar Irradiation. Environmental Science & Technology

45(24), 10385-10393.
Rubio, D., Nebot, E., Casanueva, J.F. and Pulgarín, C. (2013) Comparative effect of simulated solar light, UV, UV/H2O2 and Photo-Fenton treatment (UV-Vis/ H2O2/Fe2+,3+) in the E. coli inactivation in artificial seawater. Water Research (0).
Santos, A.L., Gomes, N.C.M., Henriques, I., Almeida, A., Correia, A. and Cunha, A. (2012) Contribution of reactive oxygen species to UV-B-induced damage in bacteria. Journal of Photochemistry and Photobiology B-Biology 117, 40-46.
Serra, J.L., Llama, M.J. and Cadenas, E. (1978) Nitrate Utilization by the Diatom Skeletonema costatum: I. Kinetics of Nitrate Uptake. Plant Physiol 62(6), 987-990.
Silverman, A.I., Peterson, B.M., Boehm, A.B., McNeill, K. and Nelson, K.L. (2013) Sunlight Inactivation of Human Viruses and Bacteriophages in Coastal Waters Containing Natural Photosensitizers. Environmental science & technology 47(4), 1870-1878.
Sinton, L., Hall, C. and Braithwaite, R. (2007) Sunlight inactivation of Campylobacter jejuni and Salmonella enterica, compared with Escherichia coli, in seawater and river water. Journal of Water and Health 5(3), 357-365.
Sinton, L.W., Hall, C.H., Lynch, P.A. and Davies-Colley, R.J. (2002) Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. Appl Environ Microbiol 68(3), 1122-1131.
Stapleton, C.M., Kay, D., Wyer, M.D., Davies, C., Watkins, J., Kay, C., McDonald, A.T., Porter, J. and Gawler, A. (2009) Evaluating the operational utility of a Bacteroidales quantitative PCR-based MST approach in determining the source of faecal indicator organisms at a UK bathing water. Water Research 43(19), 4888-4899.
Tuveson, R.W., Larson, R.A. and Kagan, J. (1988) Role of cloned carotenoid genes expressed in Escherichia coli in protecting against inactivation by near-UV light and specific phototoxic molecules. J Bacteriol 170(10), 4675-4680.
Ubomba-Jaswa, E., Fernandez-Ibanez, P., Navntoft, C., Polo-Lopez, M.I. and McGuigan, K.G. (2010) Investigating the microbial inactivation efficiency of a 25 L batch solar disinfection (SODIS) reactor enhanced with a compound parabolic collector (CPC) for household use. Journal of Chemical Technology and Biotechnology 85(8), 1028-1037.
van der Meulen, F.W., Ibrahim, K., Sterenborg, H.J., Alphen, L.V., Maikoe, A. and Dankert, J. (1997) Photodynamic destruction of Haemophilus parainfluenzae by endogenously produced porphyrins. J Photochem Photobiol B 40(3), 204-208.
Walters, S.P. and Field, K.G. (2009) Survival and persistence of human and ruminant-specific faecal Bacteroidales in freshwater microcosms. Environ Microbiol 11(6), 1410-1421.
Walters, S.P., Yamahara, K.M. and Boehm, A.B. (2009) Persistence of nucleic acid markers of health-relevant organisms in seawater microcosms: implications for their use in assessing risk in recreational waters. Water Res 43(19), 4929-4939.
Whitman, R.L., Nevers, M.B., Korinek, G.C. and Byappanahalli, M.N. (2004) Solar and temporal effects on Escherichia coli concentration at a lake Michigan swimming beach. Applied and Environmental Microbiology 70(7), 4276-4285.
Wyer, M.D., Kay, D., Watkins, J., Davies, C., Kay, C., Thomas, R., Porter, J.,

Stapleton, C.M. and Moore, H. (2010) Evaluating short-term changes in recreational water quality during a hydrograph event using a combination of microbial tracers, environmental microbiology, microbial source tracking and hydrological techniques: A case study in Southwest Wales, UK. Water Research 44(16), 4783-4795.

7. Figures and Tables

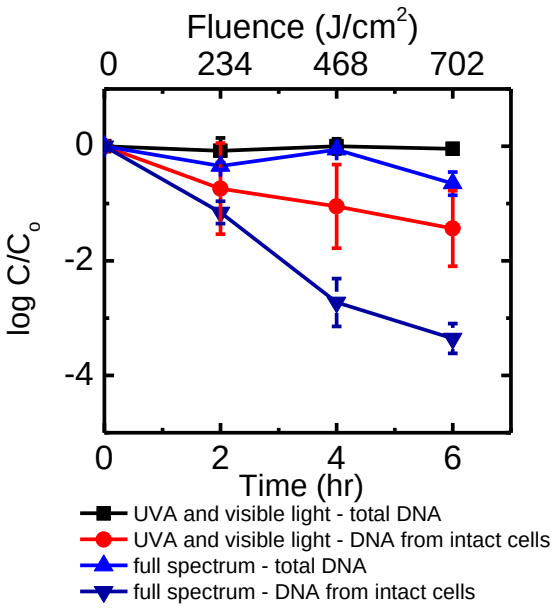

Figure 1. Comparison of the persistence of *B.ovatus* in freshwater irradiated by full spectrum simulated sunlight and UVA with visible light. Error bars correspond to standard deviation of up to 5 replicates.

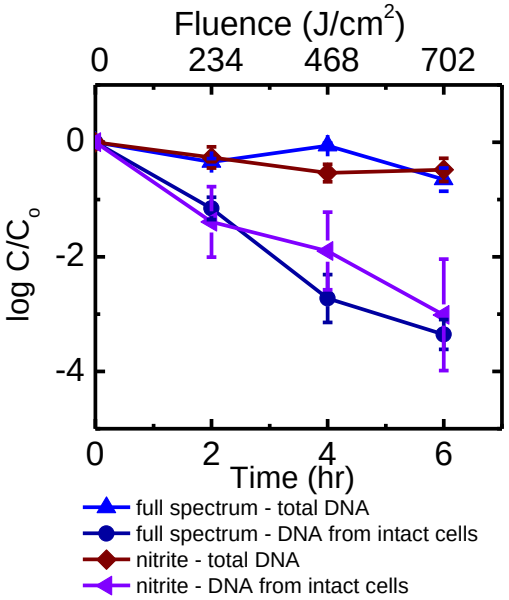

Figure 2. Comparison of the persistence of *B.ovatus* irradiated by full spectrum simulated sunlight in freshwater and 0.14mM $NaNO_2$. Error bars correspond to standard deviation of up to 5 replicates.

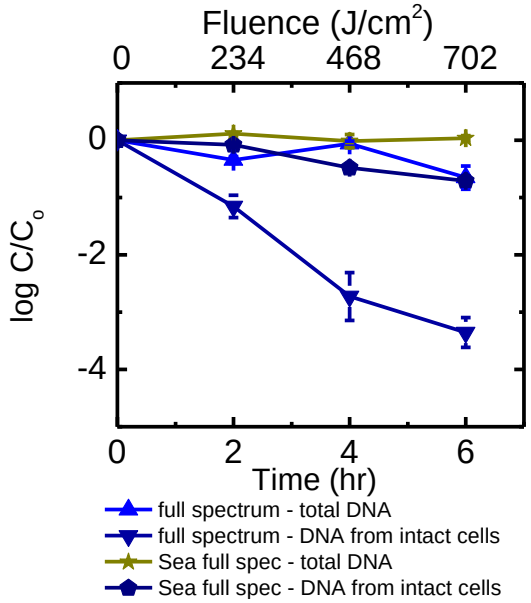

Figure 3. Comparison of the persistence of *B.ovatus* irradiated by full spectrum simulated sunlight in freshwater and artificial seawater. Error bars correspond to standard deviation of up to 5 replicates.

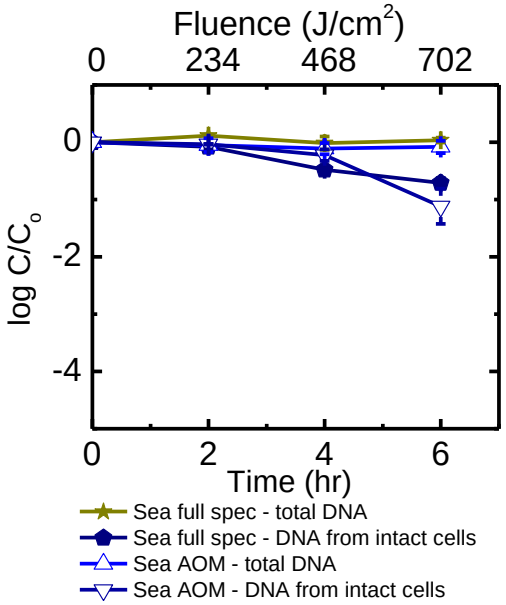

Figure 4. Comparison of the persistence of *B.ovatus* irradiated by full spectrum simulated sunlight in artificial seawater with and without AOM. Error bars correspond to standard deviation of up to 5 replicates.

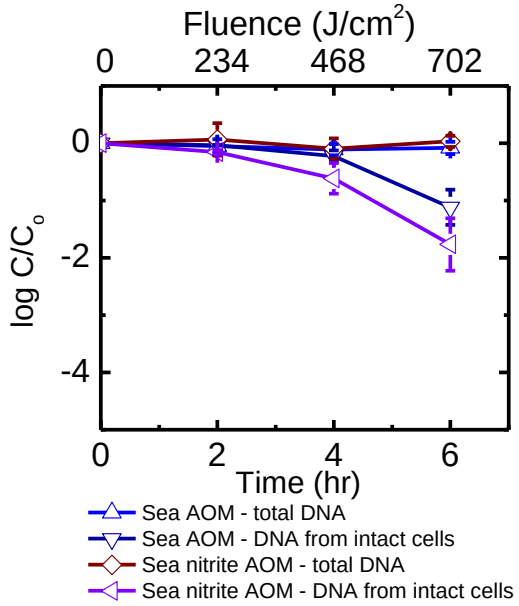

Figure 5. Comparison of the persistence of *B.ovatus* in artificial seawater irradiated by full spectrum simulated sunlight containing AOM and 0.14mM $NaNO_2$ with AOM. Error bars correspond to standard deviation of up to 5 replicates.

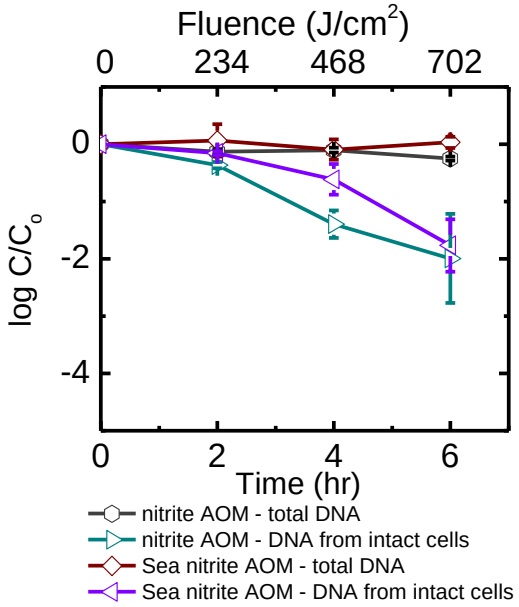

Figure 6. Comparison of the persistence of *B.ovatus* irradiated by full spectrum simulated sunlight between in artificial seawater containing AOM and 0.14mM $NaNO_2$, and that in phosphate buffer containing AOM and 0.14mM $NaNO_2$. Error bars correspond to standard deviation of up to 5 replicates.

Table 1. Decay rate constants observed for *B. ovatus*

Condition	k_{obs} in hr^{-1} and half-life in h for buffered solution	k_{obs} in hr^{-1} and in half-life in h for artificial seawater
Phosphate buffer irradiated by UVA and visible light	4.14±1.9 (0.21±0.12)	Not available
Phosphate buffer irradiated by full spectrum	9.09±0.98 (0.08±0.009)	3.32±0.38 (0.21±0.02)
Phosphate buffer dark control	0.08±0.04 (9.17±3.28)	−0.01±0.04 (4% increase)*
Phosphate buffer and $NaNO_2$ irradiated by full spectrum	6.73±1.68 (0.11±0.02)	4.22±0.25 (0.17±0.01)
Phosphate buffer and $NaNO_2$ dark control	0.06±0.04 (12.84±7.18)	0.02±0.01 (34.12±17.5)
AOM solution irradiated by full spectrum	2.06±0.33 (0.34±0.05)	4.03±1.17 (0.19±0.04)
AOM solution dark control by full spectrum	−0.02±0.04 (7% increase)*	0.04±0.01 (16.18±0.19)
$NaNO_2$ AOM solution irradiated by full spectrum	4.71±1.86 (0.17±0.05)	7.01±1.97 (0.1±0.03)
$NaNO_2$ AOM solution dark control	−0.05±0.08 (36% increase)*	−0.03±0.02 (9% increase)*

* Half-life could not be calculated due to the increase in biomass, therefore the percentage of growth was calculated.

Table 2. Hydroxyl radical concentration for different conditions

Experimental conditions	Hydroxyl radical concentration (10^{-1}fM)
Buffered freshwater + NO_2^-	7.70±0.75
Buffered freshwater + AOM	0.91±0.36
Buffered freshwater + AOM + NO_2^-	5.88±1.22
Buffered seawater + NO_2^-	3.58±1.25
Buffered sea + AOM	0.17±0.08
Buffered sea + AOM + NO_2^-	4.17±1.60

Table 3. Pyrimidine dimer counts for *B.ovatus* and antibiotic resistant bacteria (ARBs)

		Dimers (count)					Dimers/amplicon length (%)					Dimers/total dimers(%)			
Gene name	Genome size (bp)	TT	CC	TC	CT	total	TT	CC	TC	CT	total	TT	CC	TC	CT
Genome constructed from 32 scaffolds (Accession:NZ_DS2645(53-84).1)	6465369	612293	298804	413170	351734	1676001	9.5	4.6	6.4	5.4	26	36.5	17.8	24.7	21
Data on ARBs as a comparison from McKinney and Pruden, 2012															
MRSA	2872769	706446	145879	302081	766117	1431985	24.6	5.08	10.5	9.66	49.8	49.3	10.2	21.1	19.4
VRE	2826716	638915	199341	373437	318366	1530059	22.6	7.05	13.2	11.3	54.1	41.8	13.0	24.4	20.8
E.coli SMS-3-5	5068389	746734	589024	584759	517931	2438448	14.7	11.6	11.5	10.2	48.1	30.6	24.2	24.0	21.2
P.aeruginosa 01	6264404	376612	1171802	766117	707725	3022256	6.01	18.7	12.2	11.3	48.2	12.5	38.8	25.3	23.4

MRSA=methicillin-resistant *Staphylococcus aureus*, VRE=vancomycin-resistant *Enterococci*

8. Supplementary Information

What is the persistence of *B.ovatus* under simulated sunlight and in the presence of environmental conditions similar to sea water?

Question	Experiment Conducted	Findings
What are the inactivation kinetics at 27 oC under the irradiation of full spectrum UV and visible light, and UVA and visible light?	*1. B.ovatus* inactivation experiment with full spectrum UV *2. B.ovatus* inactivation experiment with UVA and visible light	2. was slower than 1., meaning that UVB was important for *B.ovatus* inactivation. Would then start to work with full spectrum only because UVA only conditions were slow
What roles do exogenous photosensitizers (NO_2^- and AOM) play in *B.ovatus*?	3. *B.ovatus* inactivation experiment in full spectrum UB and visible light in the presence of the following combinations of exogenous photosensitizers: i. $NaNO_2$ ii. AOM iii. $NaNO_2$ and AOM	i. was no different to 1., meaning that indirect exogenous inactivation for *B.ovatus* was not as important as endogenous inactivation ii. was slower than 1., indicating a protective effect offered by AOM
What's the inactivation kinetics at 27°C with full spectrum UV and visible light in artificial seawater?	4. *B.ovatus* inactivation experiment with full spectrum UV and visible light	4. was slower than 1., possibly that endogenously produced ROS got scavenged by salt (e.g. chloride ions)
How do exogenous sensitizers (NO_2^- and AOM) perform in terms of accelerating/slowing the *B.ovatus* inactivation in artificial seawater?	5. *B.ovatus* inactivation experiment with full spectrum UV and visible light in the presence of the following combinations of exogenous photosensitizers: iv. $NaNO_2$ v. AOM vi. $NaNO_2$ and AOM	1. >iv., which was consistent with the finding above, where exogenously produced hydroxyl radicals got scavenged by chloride ions in seawater, slowing down the decay. The protective effect of AOM was not as obvious as in the buffer; The fact that AOM did not speed up inactivation was consistent with findings from 3. In seawater, vi. is faster than v., suggesting a synergistic effect of AOM and NO_2^- in accelerating the decay. vi. Is also faster than iii, suggesting a synergistic effect of AOM and NO_2^- with the presence of seawater.

Figure 7. Experimental design, research questions and findings.

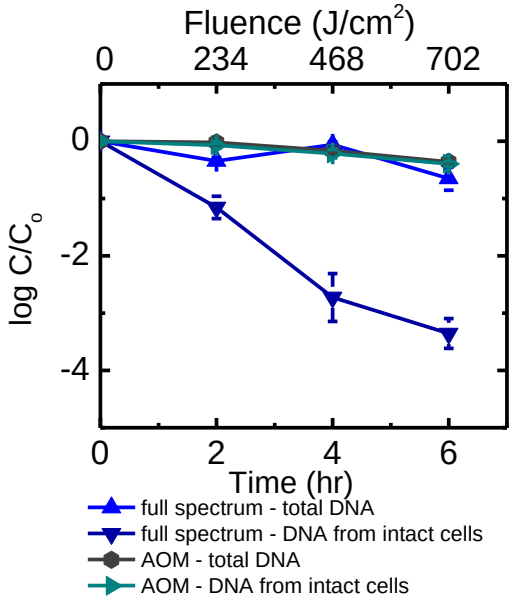

Figure 8. Comparison of the persistence of *B.ovatus* irradiated by full spectrum simulated sunlight in freshwater and AOM. Error bars correspond to standard deviation of up to 5 replicates.

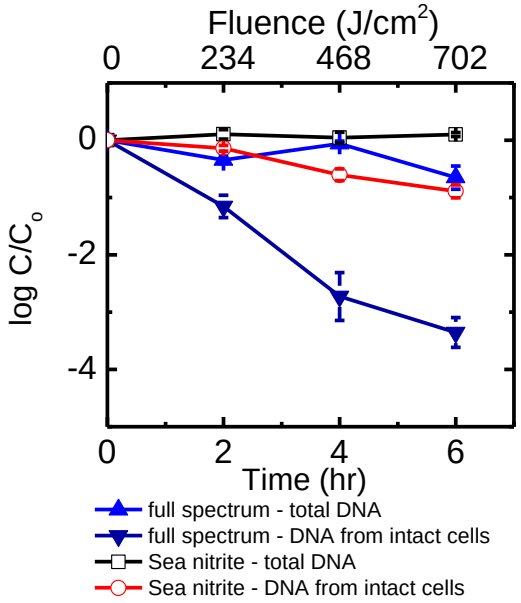

Figure 9. Comparison of the persistence of *B.ovatus* irradiated by full spectrum simulated sunlight in freshwater and artificial seawater with 0.14mM $NaNO_2$. Error bars correspond to standard deviation of up to 5 replicates.